

Genome Sequence of *Pseudomonas stutzeri* SDM-LAC, a Typical Strain for Studying the Molecular Mechanism of Lactate Utilization

Tianyi Jiang,^a Chao Gao,^{a,b} Fei Su,^b Wen Zhang,^a Chunhui Hu,^a Peipei Dou,^a Zhaojuan Zheng,^a Fei Tao,^b Cuiqing Ma,^a and Ping Xu^{a,b} State Key Laboratory of Microbial Technology, Shandong University, Jinan, People's Republic of China,^a and State Key Laboratory of Microbial Metabolism, Shanghai Jiao Tong University, Shanghai, People's Republic of China^b

Pseudomonas stutzeri SDM-LAC is an efficient lactate utilizer with various applications in biocatalysis. Here we present a 4.2-Mb assembly of its genome. The annotated four adjacent genes form a lactate utilization operon, which could provide further insights into the molecular mechanism of lactate utilization.

seudomonas stutzeri, which is widely distributed in the environment, was first described by Burri and Stutzer in 1895 (2, 9). Some strains of this species were particularly studied because of their specific functions in nature, such as nitrification, denitrification, degradation of aromatic compounds, and nitrogen fixation (9). P. stutzeri SDM-LAC (the strain was previously named SDM, CCTCC no. M206010) has received attention because of its good ability to produce pyruvate by utilizing lactate without hydrogen peroxide production detected (7). Other potential applications of this strain, such as 2-oxobutyrate production (5) and kinetic resolution of 2-hydroxy acid racemic mixtures (4, 6), have also been explored. On the basis of the results of some primary enzymological researches (11), it is thought that the enzymes that participate in lactate utilization are crucial for these applications. Thus, genetic analysis in the strain should be carried out to investigate the molecular mechanism and related key enzymes of lactate utilization.

Here, we present a draft genome sequence of strain SDM-LAC, which was obtained using the Illumina Genome Analyzer IIx system. A total of 3,909,322 filtered reads were assembled into 201 contigs using Velvet algorithms (17). Genome annotation was performed by the RAST server (1). The functional description was determined using the system for delineating clusters of orthologous genes (COG) (14). Genes encoding tRNAs and rRNAs were identified by tRNAscan-SE (10) and RNAmmer (8), respectively.

The draft genome sequence of strain SDM-LAC consists of 4,242,853 bases with a GC content of 60.4%. There are 51 predicted tRNAs in it. A total of 3,884 protein-coding sequences (CDSs) were identified with an average length of 935 bp. The coding percentage is 85.6%, and 2,893 CDSs have functional predictions. Compared with the three other sequenced *P. stutzeri* strains, A1501 (15), DSM 4166 (16), and CGMCC 1.1803 (3), 2,580 (66.4%) CDSs were identified as conserved in all four strains, and 689 (17.7%) CDSs were identified as specific ones in strain SDM-LAC by using the mGenomeSubtractor server (13).

A total of 482 subsystems were determined using the RAST server. Four adjacent genes (*lldR*, *lldP*, *lldD*, and *dld-II*) encoding a lactate-responsive regulator LldR, an L-lactate permease, an L-lactate dehydrogenase, and a predicted D-lactate dehydrogenase, respectively, were annotated in the lactate utilization subsystem. The homolog of the predicted D-lactate dehydrogenase has been proven to belong to a novel family of bacterial D-lactate dehydrogenases (12). These genes form an operon related to lactate utili

zation (12). The operon is a good model for studying the molecular mechanism of lactate utilization in bacteria. The enzymes encoded by the three putative structural genes (*lldP*, *lldD*, and *dld-II*) may play a key role in the biocatalysis processes mentioned before. However, most of the genes involved in nitrogen fixation in *P. stutzeri* A1501 (15) were not annotated in the strain SDM-LAC genome sequence. Further analysis of the genome sequence might provide other useful information for studying the molecular mechanism of lactate utilization and potential applications in biocatalysis by strain SDM-LAC.

Nucleotide sequence accession numbers. The whole-genome shotgun sequence has been deposited at DDBJ/EMBL/GenBank under accession AGSX00000000. The version described in this paper is the first version, with accession number AGSX01000000.

ACKNOWLEDGMENTS

We thank Huajun Zheng and his colleagues for genome sequencing performed at the Chinese National Human Genome Center in Shanghai, People's Republic of China.

This work was supported by grants from the National Natural Science Foundation of China (31000014, 31070062, and 30821005), China Postdoctoral Science Special Foundation (201104262), and Research Fund for the Doctoral Program of Higher Education of China (20090131110036).

REFERENCES

- 1. Aziz RK, et al. 2008. The RAST Server: rapid annotations using subsystems technology. BMC Genomics 9:75.
- Burri R, Stutzer A. 1895. Ueber Nitrat zerstörende Bakterien und den durch dieselben bedingten Stickstoffverlust. Zentralbl. Bakteriol. Parasitenkd. Abt. II 1:257–265, 350–364, 392–398, 422–432.
- 3. Chen M, et al. 2011. Complete genome sequence of the type strain *Pseudomonas stutzeri* CGMCC 1.1803. J. Bacteriol. **193**:6095.
- Gao C, et al. 2009. Enantioselective oxidation of racemic lactic acid to D-lactic acid and pyruvic acid by *Pseudomonas stutzeri* SDM. Bioresour. Technol. 100:1878–1880.
- 5. Gao C, et al. 2010. Efficient production of 2-oxobutyrate from 2-hydroxybutyrate by using whole cells of *Pseudomonas stutzeri* strain SDM. Appl. Environ. Microbiol. **76**:1679–1682.
- 6. Gao C, Zhang W, Ma CQ, Liu P, Xu P. 2011. Kinetic resolution of

Received 20 November 2011 Accepted 30 November 2011

Address correspondence to Cuiqing Ma, macq@sdu.edu.cn, or Ping Xu, pingxu@sjtu.edu.cn.

Copyright © 2012, American Society for Microbiology. All Rights Reserved. doi:10.1128/JB.06478-11

2-hydroxybutanoate racemic mixtures by NAD-independent L-lactate dehydrogenase. Bioresour. Technol. **102**:4595–4599.

- 7. Hao JR, et al. 2007. *Pseudomonas stutzeri* as a novel biocatalyst for pyruvate production from DL-lactate. Biotechnol. Lett. **29**:105–110.
- Lagesen K, et al. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res. 35:3100–3108.
- Lalucat J, Bennasar A, Bosch R, García-Valdés E, Palleroni NJ. 2006. Biology of *Pseudomonas stutzeri*. Microbiol. Mol. Biol. Rev. 70:510–547.
- Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. 25: 955–964.
- Ma CQ, et al. 2007. Membrane-bound L- and D-lactate dehydrogenase activities of a newly isolated *Pseudomonas stutzeri* strain. Appl. Microbiol. Biotechnol. 77:91–98.
- 12. Pinchuk GE, et al. 2009. Genomic reconstruction of Shewanella oneiden-

sis MR-1 metabolism reveals a previously uncharacterized machinery for lactate utilization. Proc. Natl. Acad. Sci. U. S. A. **106**:2874–2879.

- Shao YC, et al. 2010. mGenomeSubtractor: a web-based tool for parallel in silico subtractive hybridization analysis of multiple bacterial genomes. Nucleic Acids Res. 38:W194–W200.
- 14. Tatusov RL, et al. 2003. The COG database: an updated version includes eukaryotes. BMC Bioinformatics 4:41.
- Yan YL, et al. 2008. Nitrogen fixation island and rhizosphere competence traits in the genome of root-associated *Pseudomonas stutzeri* A1501. Proc. Natl. Acad. Sci. U. S. A. 105:7564–7569.
- Yu HY, et al. 2011. Complete genome sequence of the nitrogen-fixing and rhizosphere-associated bacterium *Pseudomonas stutzeri* strain DSM4166. J. Bacteriol. 193:3422–3423.
- 17. Zerbino DR, Birney E. 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res. 18:821–829.